near-isostere (cyclohexanylalanine; Cha) would introduce a subtle perturbation but still allow hexamer formation. We therefore undertook the characterization of a ChaB24-containing derivative of insulin lispro, the active component of Humalog. Native-like assembly of phenol-stabilized insulin analog hexamers was demonstrated in cobalt complexes by atomic absorption spectroscopy and explicitly visualized in zinc complexes by X-ray crystallography. ChaB24-lispro-insulin exhibits native hypoglycemic potency as measured in Lewis rats rendered diabetic by steptozotocin. Preliminary studies of pharmacodynamics in anesthetized Yorkshire pigs suggest that treatment of patients with ChaB24-lispro-insulin may benefit from curtailment of the late postprandial ''tail'' of unwanted insulin action that presently confers risk of hypoglycemia. Together, these results exemplify the potential power of unnatural amino acids to optimize the therapeutic properties of a protein central to the treatment of a human disease.

3156-Pos Board B17

Optimal Nanocarrier Design for Cancer Cell Targeting

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The side effects of therapeutic agents remain a major limiting factor in cancer therapy. Targeting of cancerous cells using drug-delivering nano-carriers has proved a promising avenue for improving agent specificity. To make further progress it is imperative to determine design principles that maximize the specificity. With this goal, we theoretically study a generalized model of drug-delivering nanocarriers binding to cancerous and healthy cells. We parametrize the phase space by endocytosis rate, association/disassociation rate and ligand number; and we investigate it for a variety of endocytosis profiles and drug delivery protocols. Our numerical and analytical results, supported by Monte-Carlo simulations, show that counter-intuitively, specificity increases by several orders of magnitude if the association rate values are close to the disassociation rate and the endocytosis rate value is relatively small. This optimal region in design space is broadly robust across endocytosis profiles and drug-delivery protocols.

Membrane Protein Function II

3157-Pos Board B18

Elucidating Elastic Network Model Robustness by Parametrization with Molecular Dynamics

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Recently, there has been a surge in elastic network model (ENM) parametrizations using molecular dynamics (MD) simulations. These simple, coarse-grained models represent proteins as beads connected by harmonic springs. The motions of this system are then elucidated by normal mode analysis. The goal of these recent parametrizations is to use MD to optimize predicted motions. In this study, we optimize many ENM functional forms using a uniform dataset containing only long MD simulations. Our results show that, across all models tested, residues neighboring in sequence adopt spring constants that are orders of magnitude stiffer than more distal contacts. In addition, the statistical significance of ENM performance varied with model resolution. We also show that fitting long trajectories does not improve ENM performance due to a problem inherent in all network models tested: they underestimate the relative importance of the most concerted motions. Finally, we characterize ENMs resilience to parametrization by tessellating the parameter space. Taken together our data reveals that choice of spring function and parameters are not vital to performance of a network model and that simple parameters can by derived ''by hand'' when no data is available for fitting, thus illustrating the robustness of the models.

3158-Pos Board B19

Exploring the Energetics of Membrane Protein Stability using a Deformable Model of the Membrane

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Experimental and computational studies have shown that cellular membranes deform to stabilize the inclusion of transmembrane (TM) proteins. Recent analysis suggests that membrane bending helps to expose charged and polar amino acids to the aqueous environment and polar phospholipid headgroups. Our previous study used elasticity theory to characterize the distortions of a membrane in the presence of a TM peptide. Here, we extend our method through the implementation of a search algorithm that identifies the membrane shape that minimizes the free energy of helix insertion. We show that our model robustly identifies the hydrophobic stretch of a TM protein and allows for large deformations to embed these stretches in the membrane. When applied to the MscL stretch activated channel, our model predicts local membrane thinning at the protein boundary that is corroborated by fluorescence and cysteine scanning experiments. We go on to show that the insertion energy for proteins containing multiple charged residues is non-additive, and the model provides a clear physical mechanism for this non-additivity, which is absent in other continuum membrane models. We predict that some highly charged S4 segments from voltage-gated K+ channels are stable in the bilayer in accordance with previous experiments and molecular dynamics simulations. Our method thus captures essential aspects of protein-membrane interactions at a small fraction of the cost of traditional molecular simulations.

3159-Pos Board B20

Microsecond Dynamics of the G-Protein Coupled Receptor Squid Rhodopsin in Atomistic Detail

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The structural similarity between many G-protein coupled receptors and their extraordinary relevance for drug development sparks significant interest in their activation mechanism and dynamics. Rhodopsins play an especially important role in experimental studies, because their activation can be triggered in a very controlled way via photoexcitation of the covalently bound cofactor retinal. The successful crystallization of intermediates along the pathway to activation, which were trapped using low temperatures and specific lipid environments, provides a detailed view of some of the initial steps occurring on the picosecond to nanosecond timescale. However, structural information for later intermediates, appearing on the microsecond to millisecond timescale, is still limited. Developments of new computational platforms now allow the challenging timescale ranging from 1-10 microseconds to be explored with atomistic computer simulations. Here we report on a set of molecular dynamics simulations of squid rhodopsin for which crystal structures of activation intermediates have begun to be available recently (e.g., 3AYM in the PDB). Our simulations start from a model of the batho state right after the photoexcitation and describe the structural evolution over a timescale of 5.5 microseconds, allowing us to observe the formation of the meta-I preactivated state featuring significant deformations of helices V and VI. Besides obtaining structural information, observing the dynamics of the structural transitions allows us to identify a reaction coordinate for the activation mechanism. By comparing simulations with different force field parameters for the retinal cofactor, we also obtain additional information on the coupling of specific degrees of freedom of the retinal to the rest of protein.

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3160-Pos Board B21

Locked on One Side Only: Computer Simulations of the Outer Membrane Efflux Duct TolC in Absence and Presence of the AcrB-Docking-Domain

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In Escherichia coli TolC functions as an efflux duct interacting with various inner membrane translocases including the multi-drug efflux pump AcrB. To investigate TolC wild-type dynamics, we performed five 150ns molecular dynamics simulations of TolC in a phospholipid / water environment. One run was extended to 300ns in two unmodified, and two modified simulations deleting four sodium ions from the periplasmic bottlenecks region in one extension and removing all NaCl in the other. Whereas the inner periplasmic bottleneck I (BNI) outlined by Asp374 remained closed, opening and closing motions in an outer bottleneck (BNII) near Gly365 were observed in all unmodified simulations. In the extended simulation a consecutive binding of two sodium ions between BNII and BNI induces BNII closure. Although the removal of four sodium ions had no impact on the closed state of BNI and BNII, the complete deletion of all NaCl caused an opening of both bottlenecks. On extracellular side we observe in all runs distinctive opening and closing motions, which might hint at a novel way of action for drugs specifically targeting the TolC interior. To gain insight into TolC-AcrB interaction, we performed five 150ns MD simulations of TolC and the AcrB docking-domain (AcrB_dd). Initially placed 1nm away from TolC and identically oriented as in the AcrAB-TolC-Symmons docking structure, AcrB_dd docked to TolC within 10ns in one run. Extending this simulation to 1μs, we found an TolC-AcrB_dd docking interface characterized by a larger contact area and a slight asymmetry not